EXTENDED REPORT

Haematopoietic and endothelial progenitor cells are deficient in quiescent systemic lupus erythematosus

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Supplementary fig 1A,B available at http://ard.bmj.com/supplemental

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Background: Systemic lupus erythematosus (SLE) is associated with a high prevalence of cardiovascular disease. Circulating endothelial progenitor cells (EPCs) contribute to vascular regeneration and repair, thereby protecting against atherosclerotic disease. EPCs are derived from CD34+ haematopoietic stem cells (HSCs), which have an increased propensity for apoptosis in the bone marrow of patients with SLE.
Aim: To determine whether circulating HSCs and EPCs are reduced in SLE, contributing to an increased cardiovascular risk.
Methods: Progenitor cells were sampled from 15 female patients with SLE in prolonged clinical remission from their disease and 15 matched healthy controls. HSC and CD34+KDR+ EPCs were quantified by flow cytometry. Annexin V staining was used to identify apoptotic cells.
Results: Patients with SLE had reduced levels of circulating CD34+ HSCs and CD34+KDR+ EPCs, associated with increased HSC apoptosis. Compared with controls, the fraction of HSCs that could be identified as EPCs was higher in patients with SLE, consistent with a primary defect of HSCs. EPC outgrowth from mononuclear cells, which depends mainly on CD34− cells, was unaffected.
Conclusions: Patients with SLE have lower levels of circulating HSCs and EPCs, even during clinical remission. The data suggest that increased HSC apoptosis is the underlying cause for this depletion. These observations indicate that progenitor cell-mediated endogenous vascular repair is impaired in SLE, which may contribute to the accelerated development of atherosclerosis.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder with a strong predisposition for atherosclerotic cardiovascular disease (CVD).[1 2] Up to a 50-fold increase in the incidence of myocardial infarction has been reported in young female patients with SLE.[3] A higher prevalence of traditional risk factors such as hypertension and dyslipidaemia cannot fully account for the increased incidence of CVD.[4] Disease-specific factors must therefore also play a role.

Dysfunction of the endothelium is pivotal in the initiation and progression of atherosclerotic CVD. SLE is associated with impaired endothelial function, partly related to enhanced endothelial cell apoptosis.[5–7] Bone marrow-derived endothelial progenitor cells (EPCs) in the circulation contribute to the maintenance and repair of the endothelium and enhance angiogenesis.[8 9] EPC levels correlate positively with endothelial function,[10] and low EPC levels were shown to independently predict cardiovascular events in patients with coronary artery disease, supporting a pathophysiological role of EPC deficiency in CVD.[11 12] EPCs constitute a bone marrow-derived subpopulation of the CD34+ haematopoietic stem cells (HSCs), identified by the coexpression of the vascular endothelial growth factor (VEGF)-receptor kinase domain receptor (KDR) as CD34+KDR+ EPC.[9] In bone marrow aspirates from patients with active SLE, increased apoptosis of HSC has been observed, which may explain the defective haematopoiesis.[13] Whether progenitor cells are also impaired during disease remissions, whether the EPC subpopulation is affected and whether this translates to reduced HSC and EPC levels in the circulation have not been reported.

We hypothesised that even during prolonged clinical remission, HSCs and consequently circulating EPCs are reduced in patients with SLE, impairing endothelial repair and thereby contributing to an increased propensity for the development of atherosclerotic disease.

METHODS

Study population

Fifteen consecutive female patients with SLE attending the lupus clinic of the University Medical Centre Utrecht, Utrecht, The Netherlands, were included in this study. Inclusion criteria were meeting at least four classical American College of Rheumatology criteria for SLE,[14] having clinically inactive disease for at least 1 year and taking a maximal daily dose of 10 mg prednisone. Exclusion criteria were current pregnancy, the use of statins and impaired renal function (Cockcroft–Gault estimated creatinine clearance of <80 ml/min). Fifteen healthy women matched for age and smoking behaviour served as controls. At the time of blood sampling, RHWMD scored clinical disease activity on a scale of 0 to 10 (0, no activity; 10, severe disease activity) and determined the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).[15] All subjects gave written consent before entering the study, which was approved by the medical ethics committee of the University Medical Centre Utrecht.

Abbreviations: 7-AAD, 7-amino-actinomycin-D; ABI, Ankle-Brachial Index; AI, Augmentation Index; CVD, cardiovascular disease; EPCs, endothelial progenitor cells; FITC, fluorescein isothiocyanate; FS, forward scatter; HSCs, haematopoietic stem cells; HUVECs, human umbilical vein endothelial cells; KDR, kinase domain receptor; PE, phycoerythrin; PWV, pulse wave velocity; SS, sideward scatter; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; VEGF, vascular endothelial growth factor

Assessment of arterial stiffness

As a surrogate marker of subclinical atherosclerosis, arterial stiffness was assessed by measuring the Ankle-Brachial Index (ABI), pulse wave velocity (PWV) and the pulse wave Augmentation Index (AI). For determination of the PWV and AI, applanation tonometry was performed using the SphygmoCor2000 device according to the manufacturer's instructions.

Flow cytometry for circulating type I EPC

EDTA blood was incubated with anti-CD34-FITC (fluorescein isothiocyanate) (BD Pharmingen, San Diego, California, USA), anti-KDR-PE (phycoerythrin; R&D Systems, Minneapolis, Minnesota, USA), and anti-CD45-PE-Cy7 (BD Pharmingen) antibodies and 7-amino-actinomycin-D (7-AAD; BD Pharmingen). Erythrocytes were lysed in an ammonium chloride buffer. CD34+KDR+ type I EPCs were quantified in duplicate from around 200 000 events per sample (average of 192 957 events; range 125 426–316 852) using a flow cytometer (Beckman Coulter, Fullerton, California, USA). For HSC and EPC identification, first the CD34+ HSCs were gated based on FITC signal and appropriate sideward scatter (SS) in the lymphocyte/monocyte range. Next, CD34+ cells were evaluated for the expression of KDR based on the presence of a concomitant PE signal. In addition, the number of $CD45+FS_{high}SS_{high}$ granulocytes in the sample was determined on a forward scatter–sideward scatter (FS–SS) plot of cells positive for panleucocyte marker CD45, which was gated on a histogram of the PE-Cy7 signals. EPC numbers per millilitre of blood were subsequently estimated based on full blood granulocyte count made using a haematocytometer. Typical examples are depicted in Supplementary fig 1A (available at http://ard.bmj.com/supplemental). Isotype-stained samples served as negative controls.

Measurement of apoptotic HSC

EDTA blood was incubated with anti-CD34-FITC and anti-CD45-PE-Cy7 antibodies. After erythrocyte lysis, cells were stained with annexinV-PE (BD Pharmingen) and 7-AAD in annexin V binding buffer (BD Pharmingen) and analysed by flow cytometry. Apoptotic HSCs were defined as $CD34+annexinV+FS_{low}$ $7\text{-}AAD_{dim}$ cells. For these analyses, around 200 000 events were also analysed per sample. A reduced FS debris threshold was used, as apoptotic cells have a decreased FS. First, CD34+ HSCs were identified and gated, followed by gating for CD34+ cells that bound annexinV. These cells were plotted on a scatterplot for their FS and 7AAD signals. CD34+annexinV+ HSCs with a low FS and an intermediate 7AAD staining were considered to be apoptotic HSCs. The intermediate 7AAD staining is named ''dim'', indicating that cells take up small amounts of the dye, which is characteristic for apoptosis. Fully viable cells with completely intact cell membranes keep all of the 7AAD out of the cell and are 7AAD negative. Dead cells take up large amounts of 7AAD, resulting in a so-called ''bright'' signal. Typical examples are depicted in supplementary fig 1B. Isotype-stained samples served as negative controls.

Type II EPC culture from mononuclear cells and functional characterisation

EPC-mediated repair depends on CD34+KDR+ circulating cells and also on a second type of EPC, which can be cultured from peripheral blood mononuclear cells. These type II EPCs are mainly derived from CD34− cells, particularly CD14+ monocytes, and are thought to be predominantly of importance for the secretion of paracrine angiogenic factors.[16–18] To obtain these EPCs, mononuclear cells were isolated from EDTA blood using Ficoll density-gradient separation (Histopaque 1077; Sigma, St Louis, Missouri, USA) and plated on fibronectin-coated plates in endothelial growth culture medium-2 medium (Cambrex, Walkersville, Maryland, USA), supplemented with 20% fetal calf serum (Invitrogen, Breda, The Netherlands), 100 ng/ml VEGF-165 (R&D Systems) and antibiotics. For characterisation of the secretion of angiogenic factors, EPCs cultured for 7 days were placed in serum-free endothelial basal medium-2 supplemented with selected aliquots (human epidermal growth factor, hydrocortisone, GA-1000, R^3-IGF-1, ascorbic acid and heparin) for 20 h, yielding an EPC-conditioned medium. Human umbilical vein endothelial cells (HUVECs; kindly provided by Adele Dijk, Utrecht, The Netherlands) were placed on matrigel (Chemicon, Ternecula, California, USA) in the EPC-conditioned conditioned media, which had been diluted to correct for EPC numbers in the original culture. The tubular structures formed by the HUVECs after 20 h were labelled with Calcein-AM (Molecular Probes) and tube length was measured using Scion Image Beta, V.4.0.3 (Scion Corporation, Frederick, Maryland, USA).[19]

VEGF ELISA

VEGF levels were measured in blood plasma and in cell culture supernatant in duplicate using a commercially available ELISA kit (R&D Systems), according to the manufacturer's instructions.

Statistical analysis

Data are expressed as mean (SEM) or median (range) and were analysed using SPSS V.11.5 and Graphpad Prism V.4.00 software. Students' t test was used to compare continuous variables and the Mann–Whitney U test for categorical variables. For regression analysis, Pearson's correlation coefficients were calculated except for multinomial values, where model I linear regression was used. A p value <0.05 was considered significant.

RESULTS

Subject characteristics

Table 1 presents the subject's characteristics. All patients had been clinically in remission for at least 1 year and disease activity was rated as low by the treating doctor on a 0 to 10 scale (median 0, range 0–2) and SLEDAI (median 2, range 0–4). Patients used immunosuppressive drugs in low dosages. Eight patients used prednisone (5.7 (5–10) mg/day; in five patients combined with azathioprine). Eight patients used hydroxychloroquine (in three patients combined with prednisone). Six patients had previously had nephritis, but did not have an impaired renal function at the time of the study. Three patients were positive for antiphospholipid antibodies, of whom two had a history of thromboembolic events. Blood pressure, body mass index and low-density lipoprotein-cholesterol were not significantly different between patients and controls. A slightly higher waist-to-hip ratio and lower high-density lipoprotein-cholesterol with associated lower apolipoprotein A1 levels were observed in patients with SLE. A history of cardiovascular disease and the presence of hypertension was more prevalent in the SLE group, although it was not statistically significant. The Framingham risk score and associated 10-year risk for coronary heart disease were not higher in the patients with SLE than in controls.

Arterial stiffness is not increased in the patients with SLE

The ABI in the patients with SLE was similar to that in controls (0.92 (0.03) vs 0.92 (0.01), $p = 0.86$). The aortic and brachial PWV were not different between groups (6.3 (0.5) vs 6.1 (0.3) m/s, $p = 0.72$ and 6.9 (0.2) vs 7.2 (0.4) m/s, $p = 0.54$,

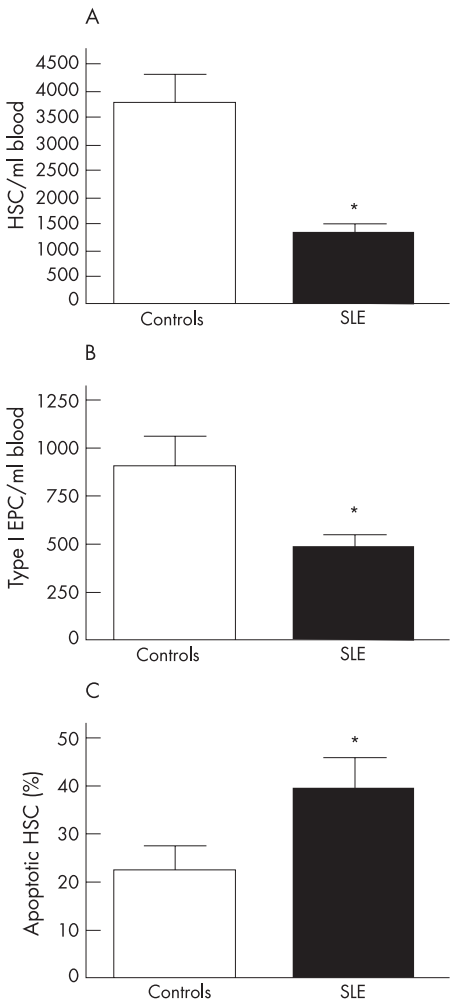

Figure 1 Circulating haematopoietic stem cell (HSC) and endothelial progenitor cell (EPC) levels. In patients with systemic lupus erythematosus (SLE), circulating CD34+ HSC (A) and CD34+KDR+ type I EPC (B) numbers were lower. This was associated with an increased fraction of apoptotic HSC, identified by 7AAD and AmexinV staining of CD34+ cells with low forward scatter (C).

respectively). Analysis of the pulse wave forms did not reveal a significant difference in the AI (24.0% (3.5%) vs 26.7% (3.0%), $p = 0.56$ and 13.9% (3.1%) vs 14.7% (4.2%), $p = 0.88$ for carotid and radial wave forms, respectively). Taken together, arterial stiffness in our population with SLE was not evidently increased in comparison with the controls.

HSCs and circulating EPCs are reduced in the patients with SLE

The total number of circulating CD34+ HSCs was lower in patients with SLE than in controls (1339 (151) vs 3813 (517)/ml blood, 65% reduction, $p<0.001$; fig 1A). There was also an absolute reduction of circulating EPC numbers in patients with SLE compared with the controls (488 (66) vs 907 (159)/ml blood, 46% reduction, $p = 0.021$; fig 1B), although the fraction of CD34+ HSC that coexpressed the EPC marker KDR was higher in patients with SLE than in controls (37% (3%) vs 25% (4%), $p = 0.015$).

HSC apoptosis is increased in patients with SLE

Patients with SLE showed more 7-AAD-positive HSCs than controls (15% (2%) vs 8% (1%), $p = 0.014$), suggestive of apoptosis. This was in contrast with the CD34− cells, the vast majority of the leucocytes, in which 7-AAD uptake was low and not significantly different between the groups (0.18% (0.02%) vs 0.18% (0.02%), $p = 0.879$). Annexin V staining confirmed a higher fraction of apoptotic CD34+ cells in patients with SLE than in controls (39.5% (6.2%) vs 22.3% (5.0%), $p = 0.0316$; fig 1C).

Mononuclear cell-derived cultured EPCs are numerically and functionally unaffected in the patients with SLE

EPC outgrowth from isolated blood mononuclear cell cultures was numerically equal in patients with SLE compared with controls (1.54 (0.3) vs 1.52 (0.4) per 100 mononuclear cells originally plated, $p = 0.97$; fig 2A, B). Stimulation of mature endothelial cell angiogenesis by EPC-conditioned medium was comparable for EPC from patients with SLE and from controls (139 (14) vs 134 (12) AU tube length, $p = 0.81$; fig 2C, D). VEGF levels, an angiogenic factor secreted by cultured EPCs in high amounts,[20] were not different in the conditioned medium, which is consistent with an intact release of paracrine proangiogenic factors (177 (38) vs 166 (34) ng secreted per well in 20 h, $p = 0.83$).

Determinants of EPC numbers

Higher ABI ($r = 0.532$, $p = 0.042$) and lower carotid ($r = -0.525$, $p = 0.045$) or radial ($r = -0.580$, $p = 0.023$) AI were associated with higher EPC levels. Cholesterol levels were inversely related to EPC numbers ($r = -0.587$, $p = 0.021$). Disease duration and activity, either scored on a clinical scale or by SLEDAI, did not show a significant correlation with circulating progenitor cell levels, although it must be noted that the limited range in the data for clinical activity and duration made correlation analysis for these parameters insensitive. There was no correlation of EPC numbers with double-stranded DNA antibody titres ($r = 0.317$, $p = 0.25$) or the levels of complement factors C3 ($r = 0.111$, $p = 0.71$) and C4 ($r = -0.008$, $p = 0.98$). The presence of antiphospolipid antibodies was also not associated with increased or decreased EPC levels (451 (154) vs 497 (76) cells/ml, $p = 0.79$). Plasma VEGF was not significantly different between patients with SLE and controls (78 (10) vs 81 (17) pg/ml, $p = 0.8976$).

The use of prednisone, regardless of the concomitant use of azathioprine, was not associated with circulating HSC numbers (1415 (233)/ml in patients using prednisone vs 1252 (198)/ml in those who did not, $p = 0.608$) or EPC levels (529 (110) vs 442 (93)/ml, $p = 0.562$). Even though patient numbers were low, hydroxychloroquine use tended to be dose dependently associated with higher EPC numbers ($r = 0.458$, $p = 0.086$ in linear regression model with 377 (74) vs 509 (136) vs 631 (138) EPCs/ml for 0 ($n = 7$), 200 ($n = 3$) and 400 ($n = 4$) mg daily doses, respectively). Furthermore, comparing patients using any dose of hydroxychloroquine ($n = 8$) with those who did not use hydroxychloroquine ($n = 7$) tended to be associated with lower arterial stiffness parameters (ABI: 0.97 (0.04) vs 0.87 (0.08), $p<0.05$; aortic PWV: 5.8 (0.4) vs 6.8 (0.8) m/s, $p = 0.308$; carotid AI: 19.2% (5.9%) vs 29.6% (1.8%), $p = 0.14$; and radial AI: 8.4% (3.6%) vs 20.2% (4.4%), $p = 0.058$) and lower cholesterol levels (4.17 (0.25) vs 4.93 (0.23), $p<0.05$).

DISCUSSION

The present study is the first to show that patients with SLE in clinical remission have decreased levels of circulating CD34+ HSCs compared with a population of matched healthy controls. Moreover, patients with SLE have reduced numbers of CD34+KDR+ EPCs, a specialised HSC subpopulation serving as progenitor cells for vascular endothelium. Our data suggest an increase in the propensity of HSCs to undergo apoptosis as

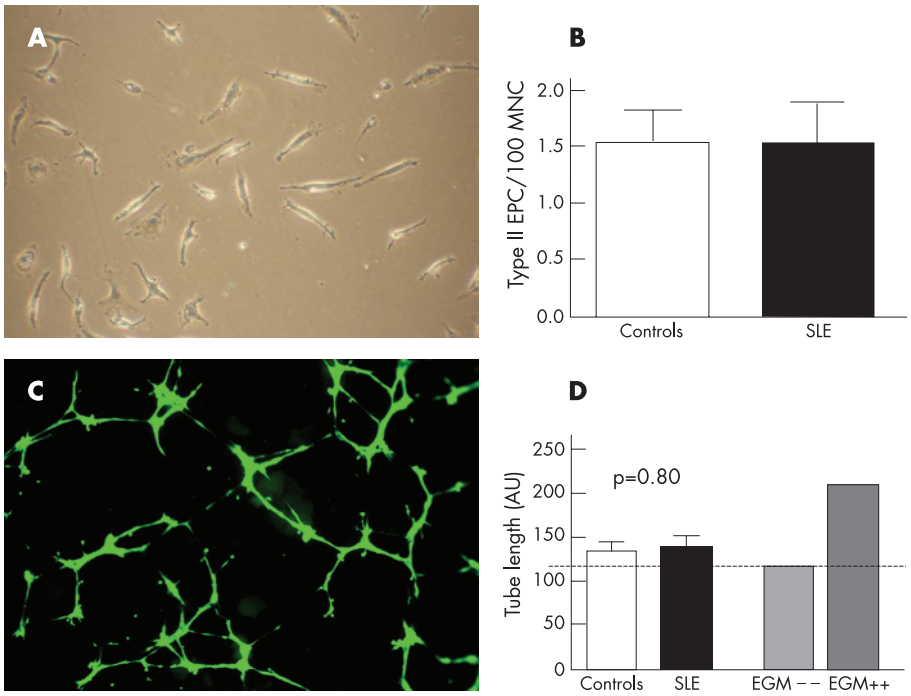

Figure 2 Endothelial progenitor cell (EPC) outgrowth from mononuclear cells in vitro and their capacity for paracrine angiogenic stimulation. EPCs cultured from peripheral blood mononuclear cells in a 7-day period gave a numerically identical outgrowth in patients with systemic lupus erythematosus (SLE) as the healthy controls (A, phase contrast at 100× magnification; B, quantification). Functionally, stimulation of human umbilical vein endothelial cell (HUVEC) angiogenesis by the EPC-conditioned medium was not statistically different (C, Calcein-AM-labelled HUVEC vascular networks on Matrigel, visualised by fluorescence microscopy at 50× magnification; D, quantification based on HUVEC tube length, with a reference line for the effect of non-conditioned blanco medium). EGM, endothelial growth culture medium; MNC, mononuclear cell.

the underlying mechanism of the lowered HSC and EPC levels. EPCs have been proposed to play a key role in endothelial repair, thereby protecting against atherosclerosis. Hence, decreased levels may contribute to the increased risk of developing CVD in patients with SLE.

Apoptosis rates of circulating cells such as lymphocytes[21] and neutrophils,[22] and also of mature endothelial cells,[7 23] have been reported to be increased in patients with SLE. One study reported increased HSC apoptosis and decreased CD34+ HSC levels in the bone marrow of patients with active SLE.[13] In the present study, 7-AAD uptake was higher in circulating CD34+ HSCs from patients with SLE. Increased annexin V binding, a phenomenon specific for apoptosis, confirmed higher levels of apoptotic circulating CD34+ HSCs in patients with SLE. Interestingly, we did not observe increased apoptosis in CD34− leucocytes in our population with inactive disease. This is consistent with a previous study, in which increased lymphocyte apoptosis was observed in patients with active disease but not with inactive SLE,[24] although others found no correlation with disease activity.[25] Our finding of increased levels of circulating apoptotic HSCs could be the result of either increased induction of apoptosis or impaired clearance of apoptotic HSC,[26] or a combination of both. The observation of reduced levels of HSCs and EPCs suggests increased induction of apoptosis, which is consistent with previous observations of induction of apoptosis of CD34+ HSC by serum from patients with SLE in vitro.[27 28] Increased HSC apoptosis has also been observed at the bone marrow level in patients with SLE.[13] We show that this is reflected in peripheral blood and affects the CD34+KDR+ EPC subpopulation. Our observations that, although absolute levels of CD34+KDR+ EPC were lower, the proportion of CD34+ HSCs expressing KDR was higher in patients with SLE compared with controls, suggest that CD34+ HSC apoptosis is the primary defect, with a consequent decrease in CD34+KDR+ EPCs. Consistently, EPC outgrowth from unselected mononuclear cells, which is known to depend mainly on CD34− cells,[17] was unaffected. Reduced HSC levels in SLE indicate that there is a limited capacity for haematopoietic renewal, which may contribute to the cytopenias observed in these patients. Reduced EPC levels suggest an impairment of endothelial regeneration, while in SLE the demand for this regeneration is higher because of excessive endothelial cell loss.

The population with SLE we investigated consisted of patients in clinical remission at the time of the study. Therefore, our findings suggest that the negative effects of the presence of SLE on EPC-mediated endothelial repair are chronic and not confined to periods of active disease in which many inflammatory intermediates and drug effects are present. The suppressive effect on progenitor cells may be partly related to the higher prevalence of several traditional risk factors in our population. We found clearly reduced levels of atheroprotective high-density lipoprotein and its major component apolipoprotein A1 in our population, as well as a higher waist:hip ratio. The limited sample size precluded stratification for the various CVD risk factors, and we could therefore not assess whether reduced EPC levels are an independent risk factor for CVD in this population. However, the calculated Framingham risk scores were low and not statistically different in patients with SLE and controls. Moreover, no significant differences in arterial stiffness assessed by ABI, PWV and AI were observed. Therefore, SLE-specific factors, which are present even during inactive disease, seem to be responsible for reducing circulating endothelial and haematopoietic progenitor cell numbers, possibly partly through effects on ''traditional'' CVD risk factors.

Serum from patients with leucopenic SLE was reported to induce HSC apoptosis and limit HSC colony-forming capacity of HSCs isolated from peripheral blood or bone marrow of healthy donors.[27 28] This may be related to autoantibody formation. One study reported binding of IgG autoantibodies to HSCs[27]; however, others found a non-IgG serum component to be the critical factor.[28] Autoantibodies from patients with SLE have been demonstrated to induce apoptosis in cultured endothelial cells,[7 23] but whether autoantibodies are also responsible for inducing apoptosis in EPCs has not been investigated. In our study, neither circulating progenitor cell levels nor progenitor cell apoptosis rates correlated with double-stranded DNA or

Table 1 Characteristics of patients with systemic lupus erythematosus and healthy controls

	Patients (n = 15)	Controls (n = 15)	p Value
Age (years)	36.6 (2.9)	36.8 (2.7)	0.95
Smoking	4/15	4/15	1.00
RR—Systolic (mm Hg)	128 (5)	128 (3)	0.99
RR—Diastolic (mm Hg)	76 (3)	77 (2)	0.82
Ankle-Brachial Index	0.92 (0.03)	0.92 (0.01)	0.86
Resting heart rate (bpm)	75 (3)	75 (3)	0.88
Weight (kg)	77.3 (5.2)	72.3 (3.0)	0.41
BMI (kg/m^2)	27.4 (2.3)	24.8 (0.9)	0.30
Waist–hip ratio	0.82 (0.02)	0.77 (0.01)	0.03*
Premenopausal	11/15	13/15	0.37
History of occlusive CVD	2/15	0/15	0.15
Hypertension†	6/15	3/15	0.24
White cell count ($\times 10^6$/ml)	6.2 (0.4)	7.4 (0.5)	0.09
Haemoglobin level (mmol/l)	8.5 (0.2)	9.2 (0.4)	0.11
Platelets ($\times 10^6$/ml)	225 (23)	267 (13)	0.11
Total cholesterol (mmol/l)	4.52 (0.19)	5.16 (0.27)	0.04*
LDL-cholesterol (mmol/l)	2.88 (0.18)	3.15 (0.19)	0.23
HDL-cholesterol (mmol/l)	1.19 (0.09)	1.52 (0.07)	0.01*
VLDL-cholesterol (mmol/l)	0.45 (0.05)	0.49 (0.04)	0.43
Triglycerides (mmol/l)	1.59 (0.17)	1.68 (0.12)	0.64
ApoA1 (mg/dl)	112 (5)	138 (5)	<0.01*
ApoB (mg/dl)	73 (4)	80 (4)	0.25
Aortic PWV (m/s)	6.3 (0.5)	6.1 (0.3)	0.72
Brachial PWV (m/s)	6.9 (0.2)	7.2 (0.4)	0.54
Carotid AI (%)	24.0 (3.5)	26.7 (3.0)	0.56
Radial AI (%)	13.9 (3.1)	14.7 (4.2)	0.88
Time from diagnosis (months)	138 (18–329)		
Clinical activity (scale 0–10)	0 (0–2)		
SLEDAI	2 (0–4)		
Antiphospholipid antibodies detectable	3/15		
dsDNA (IU/ml)	46 (21)		
C3 (g/l)	1.00 (0.05)		
C4 (g/l)	0.14 (0.01)		
Medication			
Prednisone	8/15	0/15	
Azathioprine	5/15	0/15	
Hydroxychloroquine	8/15	0/15	
Antihypertensive drugs	2/15	0/15	
Anticoagulants	3/15	0/15	
Bisphosphanates	3/15	0/15	
Supplementary calcium	7/15	0/15	
Oral contraceptives	2/15	4/15	
Framingham risk score‡	−5 (−13–11)	−4 (−14–9)	0.97
10 years risk of CHD (%)‡	1 (1–13)	1 (1–9)	0.80

AI, Augmentation Index; Apo, apoprotein; BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PWV, pulse wave velocity; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; VLDL, very low-density lipoprotein.
*$p<0.05$.
†Hypertension was defined as having a systolic blood pressure of ⩾140 mm Hg, a diastolic blood pressure of ⩾90 mm Hg or use of antihypertensive drugs.
‡Calculated using the Coronary Disease Risk Prediction Score Sheet for Women (see http://www.nhlbi.nih.gov/about/framingham/riskwom.pdf).

antiphospholipid autoantibody titres. Increased CD34+ HSC cell apoptosis as the underlying mechanism for EPC deficiency may not only occur in SLE but also in other autoimmune diseases. In patients with rheumatoid arthritis, the rate of HSC apoptosis in bone marrow aspirates was also enhanced.[29] In other pro-atherosclerotic conditions with reduced HSC and EPC levels—that is diabetes and renal failure—progenitor cell apoptosis was not significantly increased.[19 30]

EPC-mediated repair depends on CD34+KDR+ circulating EPCs and also on a second type of EPC, which can be cultured from peripheral blood mononuclear cells. These cultured EPCs have also been reported to be reduced in the presence of traditional CVD risk factors.[10] We found outgrowth of these EPCs to be unaffected both numerically and functionally in our study. This may be consistent with observations that these EPCs are mainly derived from CD34− cells, particularly CD14+ monocytes,[16–18] whereas our observations in SLE suggest a defect in the CD34+ HSCs in a quiescent disease state.

Our study was not set up to evaluate the effect of drug treatment, and the cross-sectional design and limited sample size issue a need for caution in the interpretation of observed associations with the use of particular drugs. We observed higher levels of EPCs in patients using hydroxychloroquine. The use of hydroxychloroquine was associated with significantly lower total cholesterol and less arterial stiffness. This is consistent with previous reports on reduced cholesterol levels in patients with SLE using hydroxychloroquine.[31 32]

In conclusion, circulating CD34+ HSCs and CD34+KDR+ EPCs are markedly reduced in patients with SLE even when the disease is in clinical remission and despite the patients receiving standard treatment. HSC apoptosis is increased, which may be the underlying cause of the observed decreased

progenitor cell levels. The observed enhanced HSC apoptosis and EPC deficiency may identify a novel pathophysiological component underlying the increased risk of CVD observed in patients with SLE. Prevention of EPC apoptosis and increasing EPC levels may offer a new therapeutic approach to prevent premature atherosclerosis and CVD in patients with SLE independent of the disease activity.

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Competing interests: None declared.

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